

WHAT IS CLAIMED:

1. A method for measuring transferase enzymatic activity comprising:

(a) providing a reagent comprising a transferase quenching agent, a luminogenic molecule and a bioluminescence-generating enzyme, wherein the transferase quenching agent selectively stops transferase activity without substantially affecting bioluminescent enzyme activity;

(b) incubating a first reaction mixture comprising a transferase, ATP, and a transferase substrate for a first predetermined time period under conditions effective to allow for a transferase reaction to occur;

(c) contacting the first reaction mixture with the reagent to form a second reaction mixture and incubating the second reaction mixture for a second predetermined time period under conditions effective to allow for a bioluminescent reaction to occur; and

(d) determining transferase activity by measuring luminescence of the second reaction mixture.

2. A method for screening a compound for its effect on transferase enzymatic activity comprising:

(a) providing a compound for screening;

(b) providing a reagent comprising a transferase quenching agent, a luminogenic molecule and a bioluminescence-generating enzyme, wherein the transferase quenching agent selectively stops transferase activity without substantially affecting bioluminescence-generating enzyme activity;

(c) incubating a first reaction mixture comprising a transferase, ATP, a transferase substrate, and the compound for a first predetermined time period under conditions effective to allow for a transferase reaction to occur;

(d) contacting the first reaction mixture with the reagent to form a second reaction mixture and incubating the second reaction mixture for a second predetermined time period under conditions effective to allow for a bioluminescent reaction to occur; and

(e) determining the effect of the compound on transferase activity by measuring and comparing luminescence of the second reaction mixture relative to a control mixture having no compound.

5                    3.        A high throughput method for rapidly screening a plurality of compounds to determine their effect on transferase enzymatic activity comprising:

(a) providing a plurality of compounds for screening;

10                    (b) providing a reagent comprising a transferase quenching agent, a luminogenic molecule and a bioluminescent enzyme, wherein the transferase quenching agent selectively stops transferase activity without substantially affecting bioluminescent enzyme activity;

15                    (c) incubating a plurality of first reaction mixtures, each first reaction mixture comprising a transferase, ATP, a transferase substrate, and at least one compound, for a first predetermined time period under conditions effective to allow for transferase reactions to occur;

(d) contacting for a second predetermined time period the first reaction mixtures with the reagent to form a plurality of second reaction mixtures under conditions effective to allow for bioluminescent reactions to occur; and

20                    (e) determining the effect of the compounds on transferase activity by measuring and comparing luminescence of the second reaction mixtures relative to at least one control mixture having no compound.

25                    4.        The method of any one of claims 1, 2, or 3 wherein the transferase enzymatic activity comprises kinase activity or ion channel/pump activity.

5.        The method of claim 4 wherein the transferase enzymatic activity comprises protein kinase activity, lipid kinase activity, polynucleotide kinase activity, or sugar kinase activity.

30                    6.        The method of claim 5 wherein the protein kinase comprises a Ser/Thr protein kinase, a protein tyrosine kinase, or a protein lipid-dependent kinase.

7. The method of claim 5 wherein the Ser/Thr protein kinase comprises cAMP-dependent protein kinase (PKA), calcium and phospholipids dependent protein kinase (PKC), cGMP-dependent protein kinase (PKG), calcium and calmodulin dependent protein kinase (CaM KII) or a dual specificity protein kinase.

5

8. The method of claim 6 wherein the dual specificity protein kinase comprises mitogen activated protein kinase(MAPK) or mitogen activated protein kinase kinase(MEK).

10

9. The method of claim 6 wherein the tyrosine kinase comprises Rous sarcoma related protein kinases(Src), or Src family protein tyrosine kinases.

10. The method of claim 9 wherein the Src family protein tyrosine kinases comprise Src, Lck, Fyn, or Lyn.

15

11. The method of claim 9 wherein the growth factor receptors comprise epidermal growth factor receptor(EGFR), platelet derived growth factor receptor(PDGFR) or steel growth factor receptor(c-KIT).

20

12. The method of claim 6 wherein the lipid dependent protein kinases comprises Type I phosphoinositide 3-OH phosphatidylinositol kinase (PI3K).

13. The method of claim 12 wherein the transferase quenching agent comprises a detergent.

25

14. The method of claim 12 wherein the detergent comprises a cationic detergent, anionic detergent, or zwitterionic detergent.

30

15. The method of claim 14 wherein the cationic detergent comprises dodecyltrimethylammonium bromide, cetyltrimethylammonium bromide and benzyldimethyldodecylammonium bromide.

16. The method of claim 15 wherein the cationic detergent is dodecyltrimethylammonium bromide.
- 5 17. The method of claim 14 wherein the anionic detergent comprises SDS and deoxycholate.
17. The method of claim 14 wherein the zwitterionic detergent comprises sulfobetaine 3-10.
- 10 19. The method of any one of claims 1, 2, or 3 wherein the transferase quenching agent comprises a metal chelating agent.
20. The method of claim 19 wherein the transferase quenching agent comprises EDTA or EGTA.
- 15 21. The method of any one of claims 1, 2, or 3 wherein the transferase inhibitor comprises staurosporine, or a peptide or protein inhibitor.
- 20 22. The method of any one of claims 1, 2, or 3 wherein the luminogenic molecule comprises D-luciferin or a luciferin derivative and the bioluminescent enzyme comprises a luciferase.
23. The method of claim 19 wherein the luciferase comprises a thermostable luciferase.
- 25 24. The method of any one of claims 1, 2, or 3 wherein the transferase itself is being phosphorylated or the transferase substrate is being phosphorylated.
- 30 25. The method of any one of claims 2 or 3 wherein the compound enhances transferase enzymatic activity.

26. The method of any one of claims 2 or 3 wherein the compound inhibits transferase enzymatic activity.

27. A kit for measuring transferase enzymatic activity comprising:

- 5 (a) a reconstitution buffer solution comprising one or more transferase quenching agents, wherein the transferase quenching agent selectively stops transferase activity without substantially affecting bioluminescent-generating enzyme activity;
- 10 (b) a composition comprising a luminogenic molecule and a bioluminescence-generating enzyme; and
- (c) directions for using the kit.

15 28. The kit of claim 27 wherein the transferase enzymatic activity comprises kinase activity or ion channel/pump activity.

29. The kit of claim 28 wherein the transferase enzymatic activity comprises protein kinase activity, protein lipid-dependent kinase activity, 20 polynucleotide kinase activity, or sugar kinase activity.

30. The kit of claim 5 wherein the protein kinase comprises a Ser/Thr protein kinase, a protein tyrosine kinase, or a protein lipid-dependent kinase.

25 31. The kit of claim 30 wherein the Ser/Thr protein kinase comprises cAMP-dependent protein kinase (PKA), calcium and phospholipids dependent protein kinase (PKC), cGMP-dependent protein kinase (PKG), calcium and calmodulin dependent protein kinase (CaM KII) or a dual specificity protein kinase.

30

32. The kit of claim 31 wherein the dual specificity protein kinase comprises mitogen activated protein kinase(MAPK) or mitogen activated protein kinase kinase(MEK).

5 33. The kit of claim 30 wherein the tyrosine kinase comprises Rous sarcoma related protein kinases(Src), or Src family protein tyrosine kinases.

34. The kit of claim 33 wherein the Src family protein tyrosine kinases comprise Src, Lck, Fyn, or Lyn.

10

35. The kit of claim 33 wherein the growth factor receptors comprise epidermal growth factor receptor(EGFR), platelet derived growth factor receptor(PDGFR) or steel growth factor receptor(c-KIT).

15

36. The kit of claim 30 wherein the lipid dependent protein kinases comprises Type I phosphoinositide 3-OH phosphatidylinositol kinase(PI3K).

37. The kit of claim 26 wherein the transferase quenching agent comprises a detergent.

20

38. The kit of claim 37 wherein the detergent comprises a cationic detergent, anionic detergent, or zwitterionic detergent.

25

39. The kit of claim 38 wherein the cationic detergent comprises dodecyltrimethylammonium bromide, cetyltrimethylammonium bromide and benzyldimethyldodecylammonium bromide.

40. The kit of claim 38 wherein the cationic detergent is dodecyltrimethylammonium bromide.

30

41. The kit of claim 38 wherein the anionic detergent comprises SDS and deoxycholate.

42. The kit of claim 38 wherein the zwitterionic detergent comprises sulfobetaine 3-10.

5 43. The kit of claim 26 wherein the transferase quenching agent comprises a metal chelating agent.

44. The kit of of claim 43 wherein the transferase quenching agent comprises EDTA or EGTA.

10

45. The kit of claim 26 wherein the transferase inhibitor comprises staurosporine, or a peptide or protein inhibitor.

15 46. The kit of claim 26 wherein the luminogenic molecule comprises D-luciferin or a luciferin derivative and the bioluminescent enzyme comprises a luciferase.

47. The kit of claim 46 wherein the luciferase comprises a thermostable luciferase.

20